

EXPERIMENTAL RESEARCHES ON THE BACTERIOLOGY OF LEPROSY

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(Received for publication 30 March, 1916)

This is a brief account of the results of experiments in connection with the bacteriology of leprosy, which were begun in 1911. For the benefit of English readers, who may not have been able to follow my previous papers in Italian and German, I may state that they contain fuller descriptions and details, together with coloured plates, drawings and photographs, showing the various conditions which may arise after the inoculation of rabbits with leprosy material.

Two rabbits were inoculated with small pieces of a leprosy nodule from a private patient on August 16th, 1911; the material was grafted into the anterior chamber of the eye. In both animals the implantation was successful, and on the 3rd December, 1911, the cornea was incised, and a microscopical examination having shown numerous acid-fast micro-organisms, four other rabbits were inoculated in the same fashion with small pieces taken from the first two animals; of the four only two gave a positive result, that is the inoculation was followed by the production of corneal and iritic nodules. With material from these animals, three other rabbits were inoculated on February 25th, 1912, this being, therefore, the third transplantation or transmission. Two failed to show any permanent lesions, but the third rabbit developed in the course of three months a small grey nodule at the site of the graft, which was surrounded by several minute grey and pink granules which spread over the iris, in addition to a cloudy area on the cornea in the region of the inoculation. This opacity showed very clearly numerous arterioles, which taking their origin from the outer hazy margin reached the centre of the cloudy oval patch. These lesions gradually disappeared after two months' time, and eventually the only traces

to be detected consisted in deformity of the pupil with adhesions, diminution of the anterior chamber and localized opacity of the cornea.

After these initial experiments many attempts have been made with material from eight lepers and numerous rabbits. The leprotic lesions were successfully transferred from the first to the second and third generation, but by no means with uniform results; still I have come to the conclusion that, without varying the original method of inoculation, one can produce leprous lesions in the eye of rabbits in two out of three experiments, the second passage is usually only achieved in one out of four attempts, whilst the third removal shows a still smaller proportion of success, though even then by dealing with big series it is possible to prove that inoculation has taken place with living bacteria.

Concurrently to these experiments, very numerous and repeated attempts were made to obtain an artificial culture of the bacterium of leprosy on laboratory media, but all efforts, with one single exception, were sterile, though many different media were employed, both aerobically and anaerobically.

Once only, from a rabbit which had been inoculated 128 days previously, I succeeded in isolating an acid-fast micro-organism, identical in its morphology with Hansen's bacillus, and which I consider to be similar to the bacterium cultivated by Kedrowsky from human lepromas, though possibly my strain may differ slightly in some of its characters and properties from the Russian prototype. The culture was obtained on a single tube of egg-yolk-agar which was anaerobically incubated during a month at 37° C. Two minute wrinkled colonies were just visible, the size of pin-heads, with a peculiar pale yellow wrinkled surface; microscopically these colonies were found to consist of acid-fast bacilli, mostly in thickly matted groups similar in disposition to Hansen's bacillus as seen in lepromas; individual rods were beaded and appeared to consist of a row of extremely acid-fast granules, a feature which is also peculiar to the bacillus of leprosy. Later sub-cultures grew much more rapidly and became adapted to aerobic conditions, and could be made to thrive on most of the usual laboratory media. The optimum temperature is 37° C. The resemblance to *B. tuberculosis* is very remarkable, but this culture is easily identified by injection into

guinea-pigs. Old cultures have a tendency to lose their acid-fast properties and show numerous clubbed shapes. Young cultures (10 days) on glycerine-broth show clumps and radiate arrangement which renders them very similar to the streptothrix group.

Injections of emulsions of this bacterium into the anterior eye-chamber of rabbits have been constantly negative; a parallel experience to that obtained by injecting ground-up lepromas in the same fashion. White mice, guinea-pigs, rabbits and two monkeys have been subcutaneously and intra-peritoneally injected with emulsions of this culture, but so far only temporary, localized lesions have been obtained, if at all.

These investigations are being continued, for much remains as yet to be done; still, at the present stage they appear to me to allow the statement to be made, that, under definite experimental conditions, leprosy can be communicated to rabbits, and that a bacterium similar to that obtained by Kedrowsky can be isolated in pure culture from the experimental lesions of the eye of the rabbit.

Similar results have been achieved by Serra, Chirivino, Bayon and others, but in any case the experimenter must expect to have to face numerous incomplete results or negative inoculations and sterile culture tubes. The impatient observer will easily adopt the opinion of those authors who consider that the experimental transmission of leprosy or the artificial cultivation of its bacillus is impossible and has never succeeded.

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